

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
SUBSTRATES FOR SYNTHESIS OF TRANSCRIPTION PRODUCTS
CORRESPONDING TO TARGET SEQUENCES

Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
Lucia B. Rothman-Denes/Svetlana Y. Gerdes

Application No.:

Docket Number: 310307.00003

1/25

Bacteriophage N4 vRNAP promoters

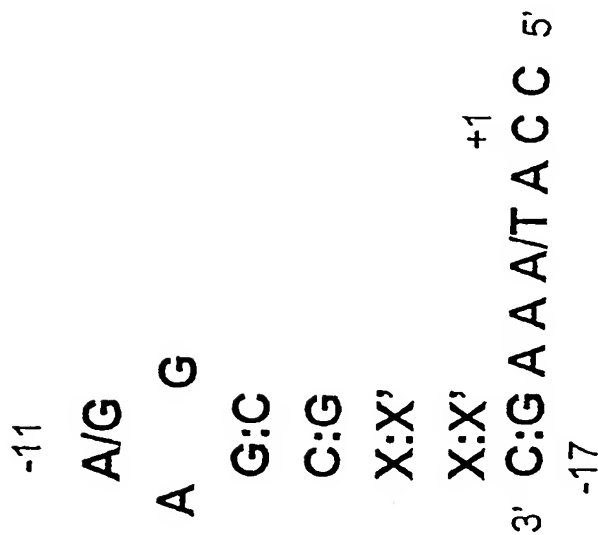


FIG. 1

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Docket Number: 310307.00003

2/25

N4 vRNAP and generation of mini-vRNAP

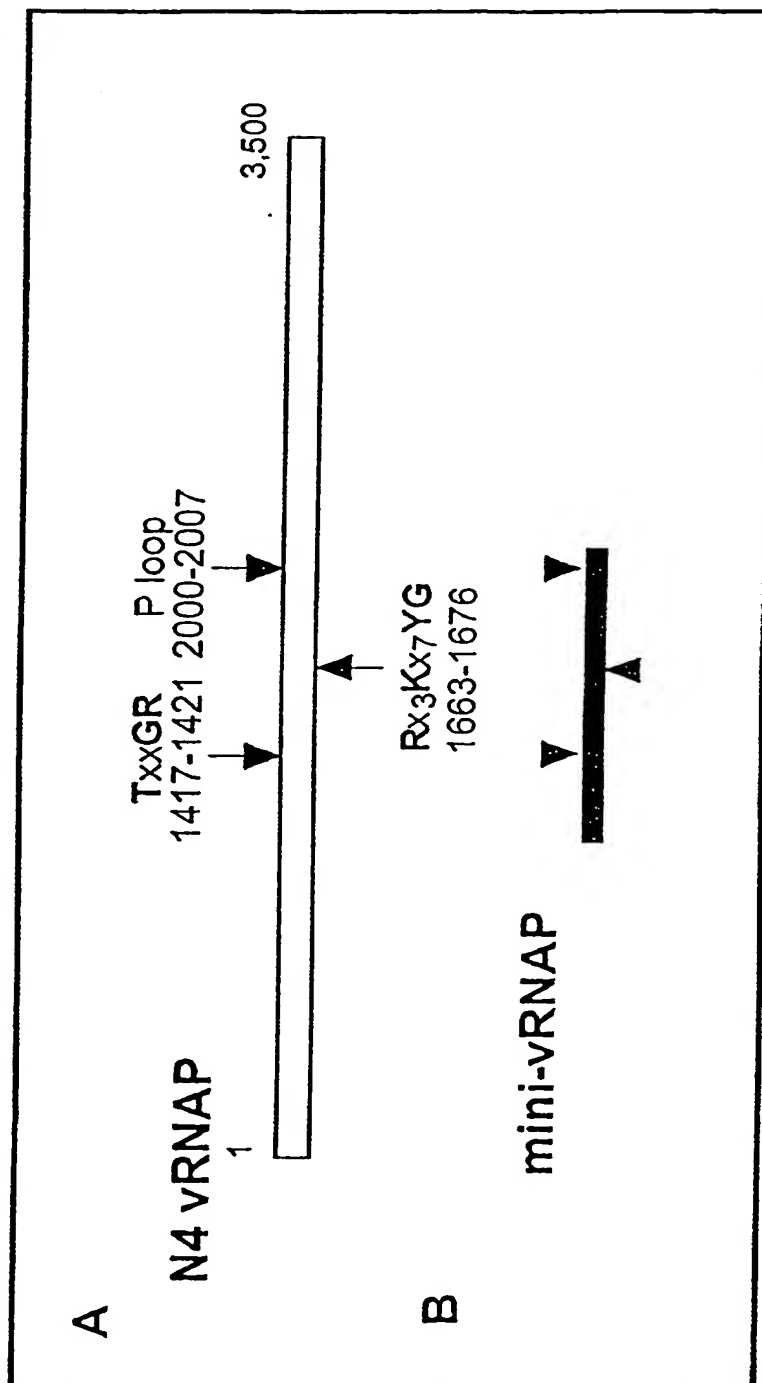


FIG. 2

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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3/25

Identification of the minimal active domain of N4 vRNAP
by proteolytic cleavage.

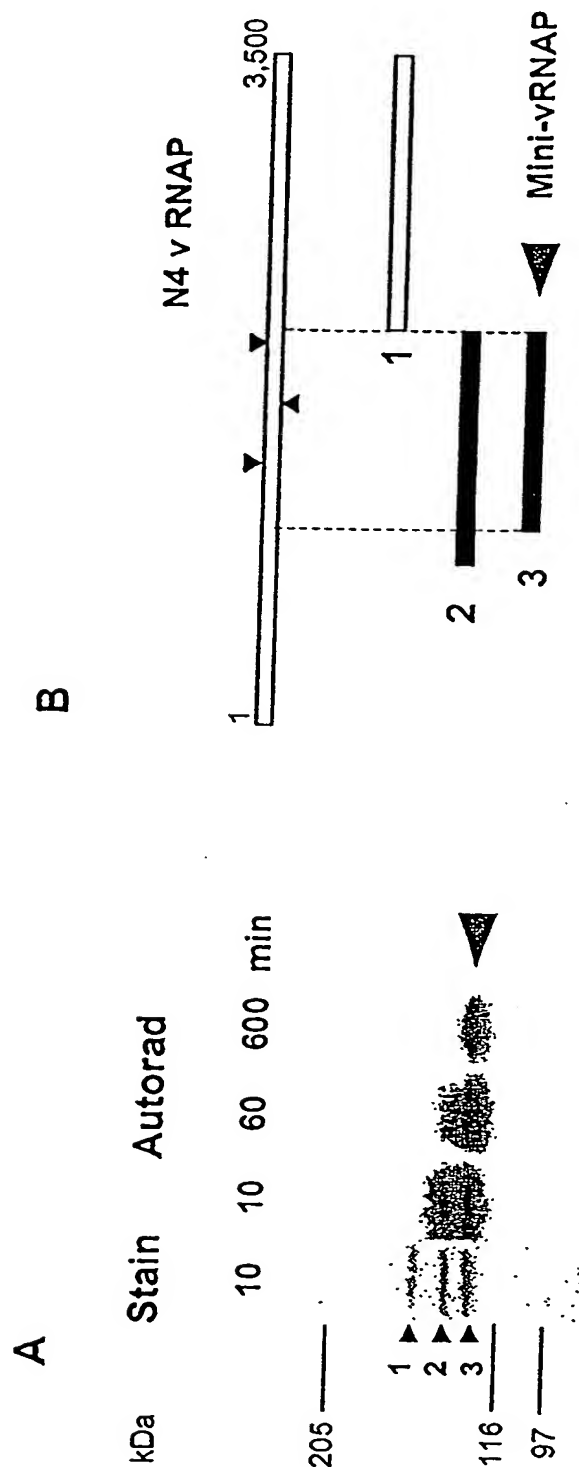


FIG. 3

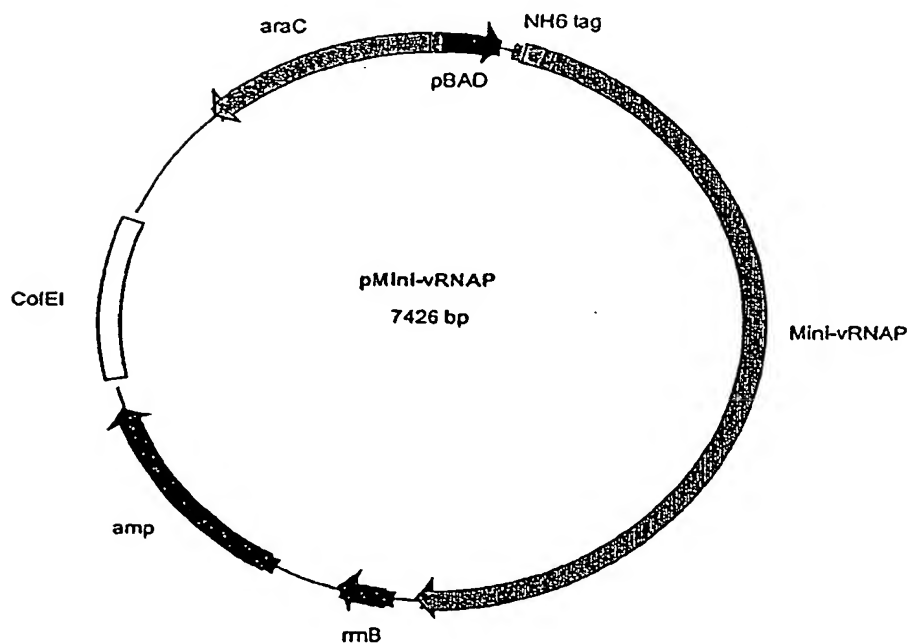
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Docket Number: 310307.00003

4/25



Plasmid name: pMini-vRNAP
Plasmid size: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD 8 expression plasmid

FIG 4

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Application No.:

Docket Number: 310307.00003

5/25

Purification of cloned vRNAP and mini-vRNAP

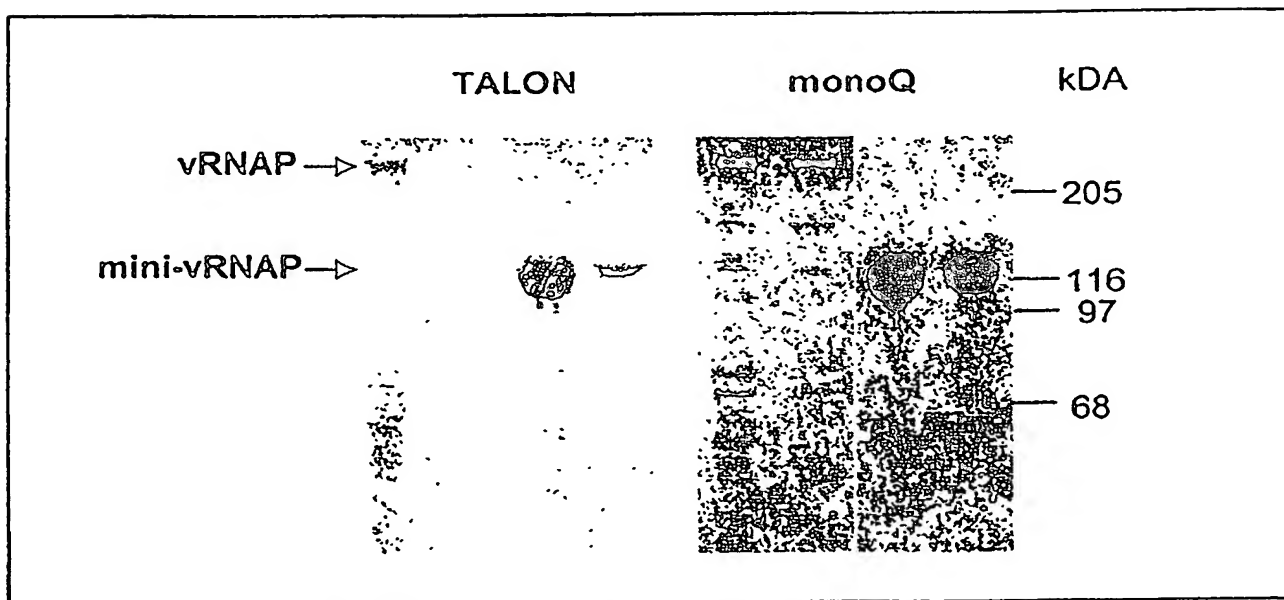


FIG 5

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Application No.:

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6/25

Activation of N4 vRNAP transcription by *Eco* SSB
at different ssDNA concentrations

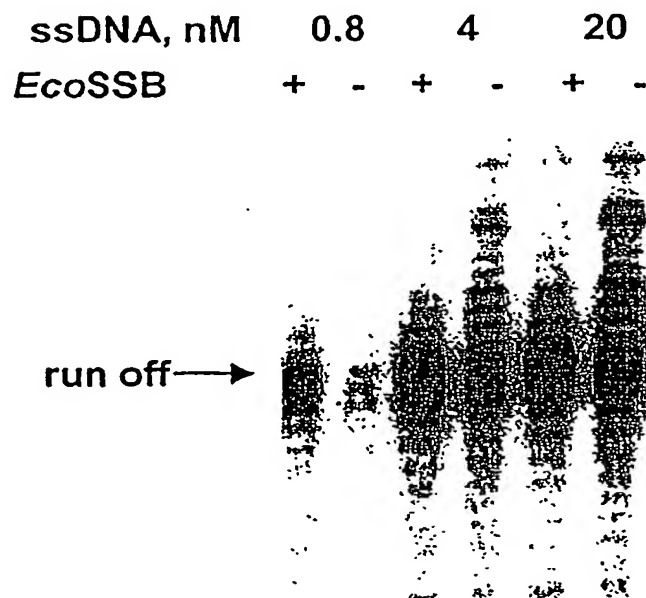


FIG 6

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Application No.:

Docket Number: 310307.00003

7/25

Effect of Eco SSB on ssDNA template recycling

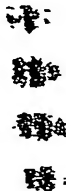
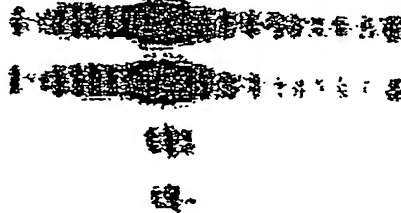
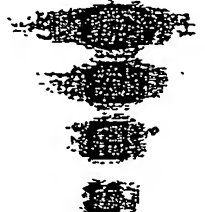
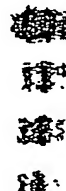
	A	B	C	D
Extra ssDNA	-	+		
Eco SSB	-	-	+	+
time at 37°C	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'
				

FIG. 7

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Application No.:
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8/25

Effect of *Eco* SSB on the state of template DNA
and product RNA in vRNAP transcription

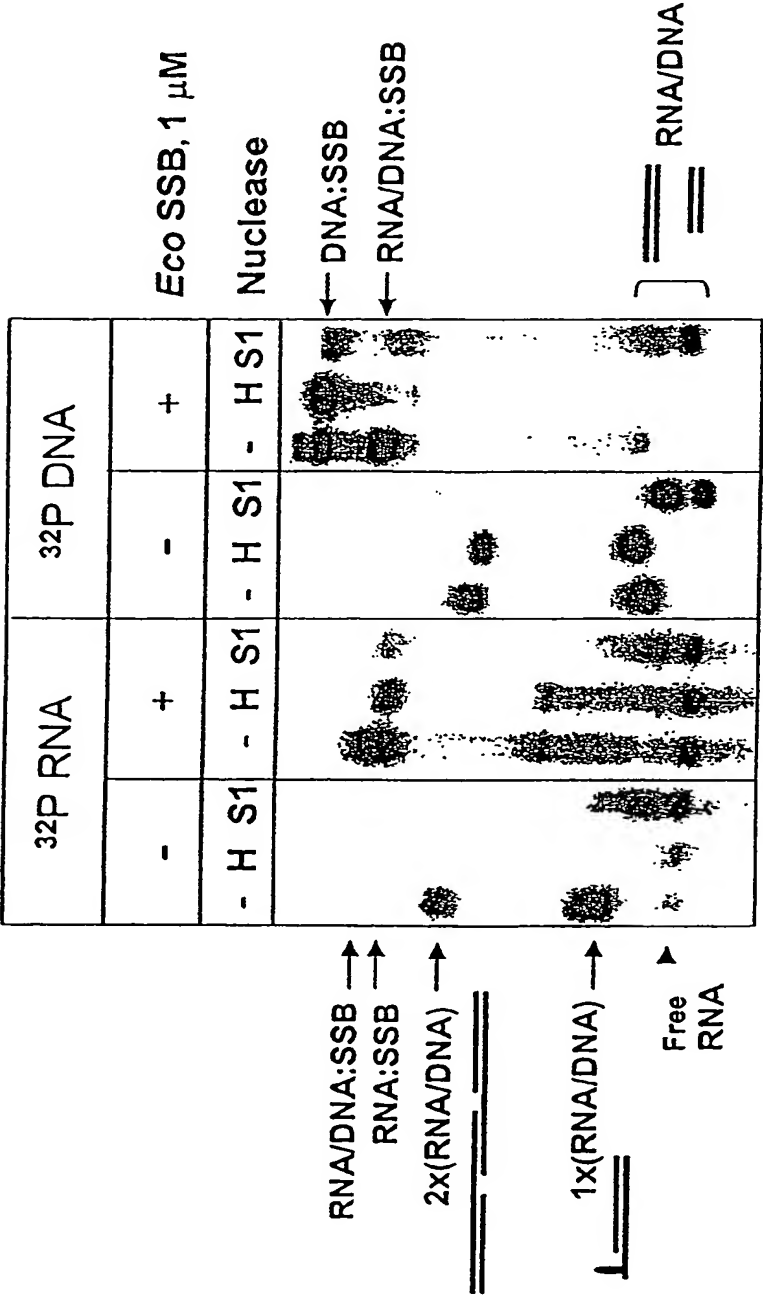


FIG. 8

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Application No.:

Docket Number: 310307.00003

9/25

Transcription initiation by vRNAP and mini-vRNAP

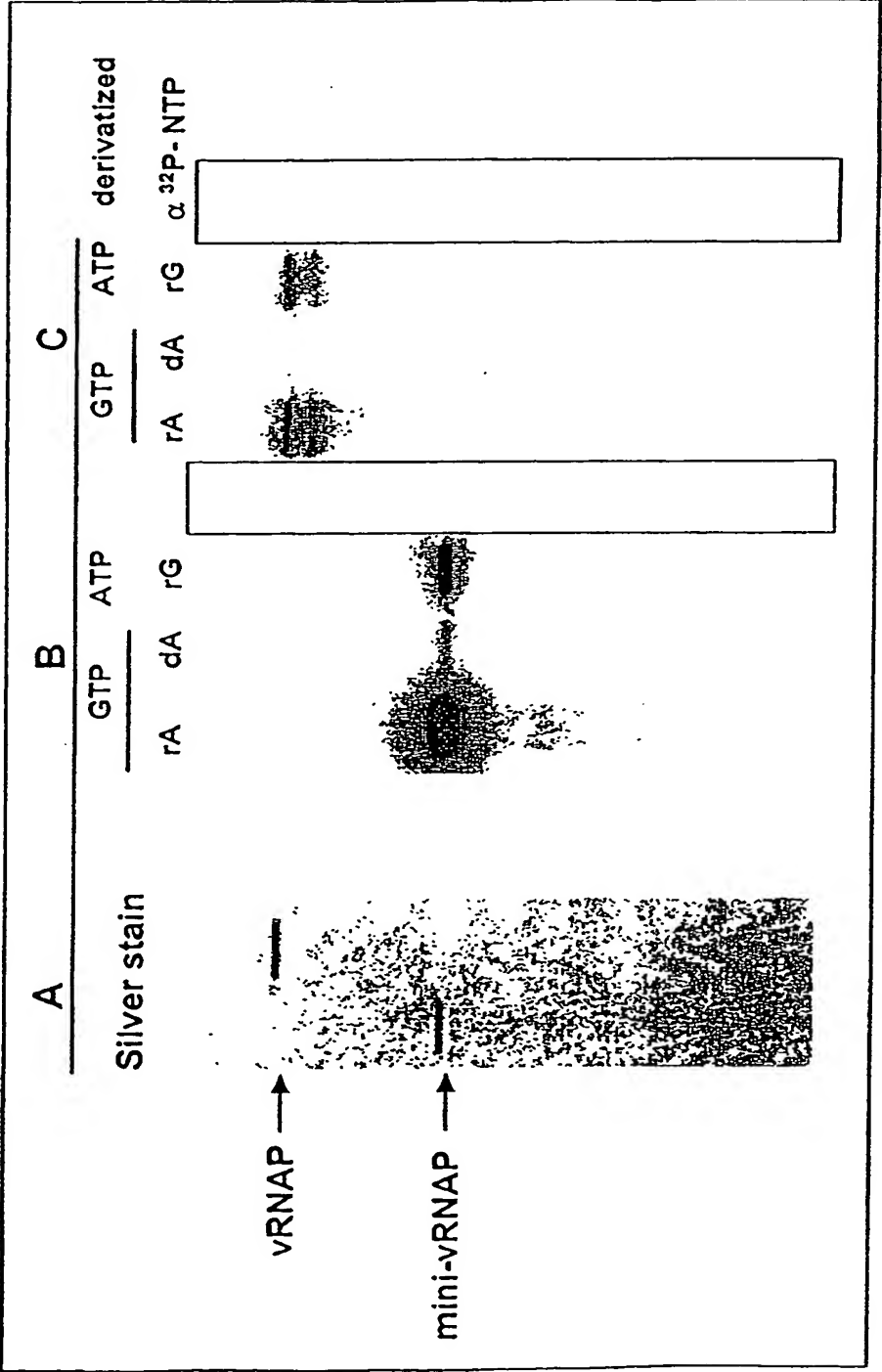


FIG. 9

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Application No.:

Docket Number: 310307.00003

10/25

Effect of *Eco* SSB on transcription of vRNAP and mini-RNAP

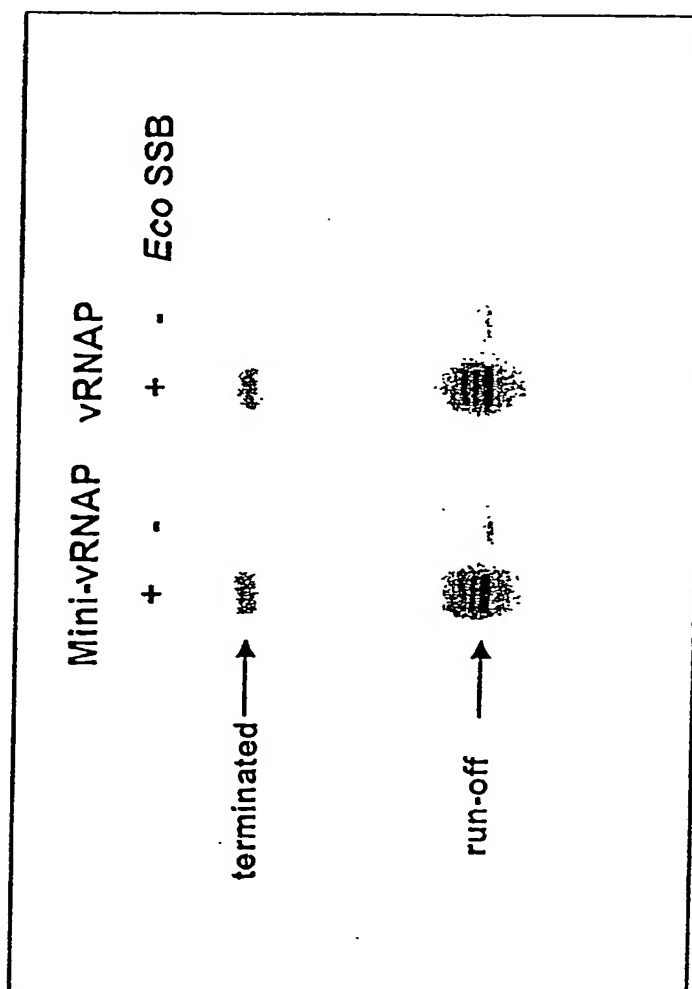


FIG. 10

**Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
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Docket Number: 310307.00003

Determination of mini-vRNAP promoter contacts

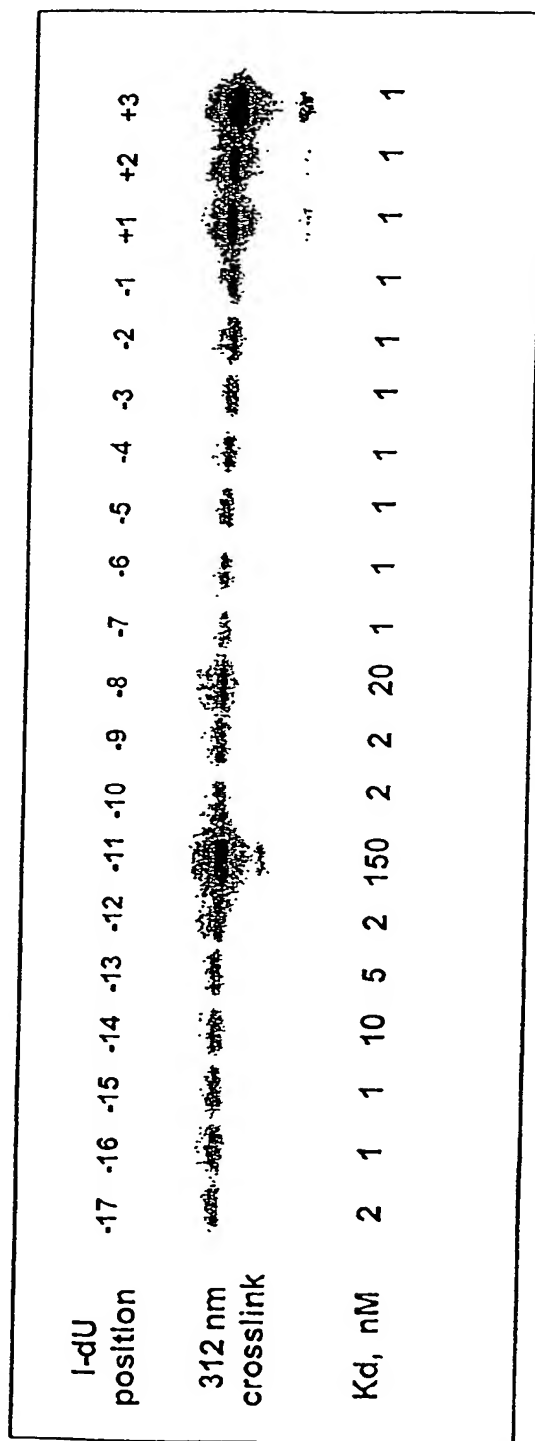
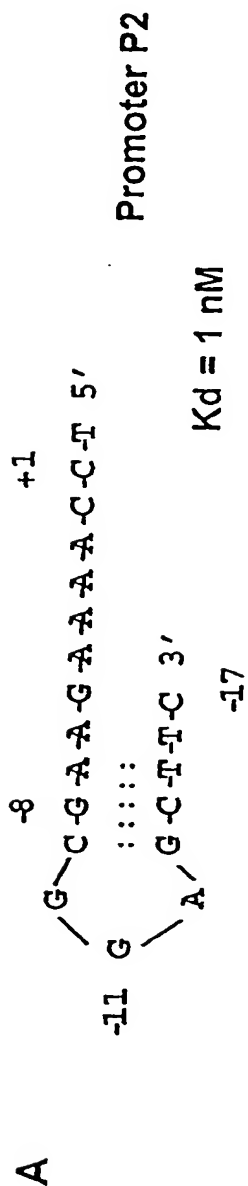


FIG. 11

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Application No.:

Docket Number: 310307.00003

12/25

Binding affinities of stem-length promoter mutants

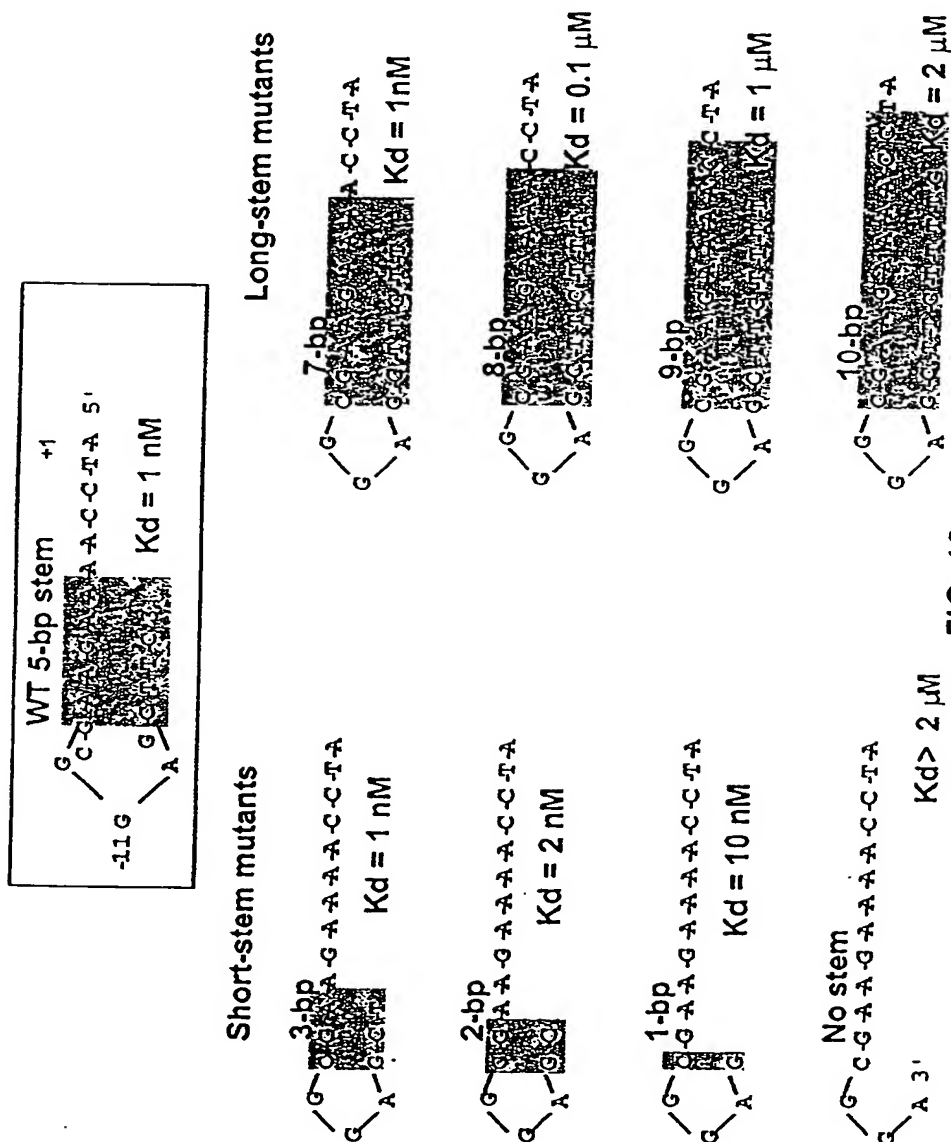


FIG. 12

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Application No.:

Docket Number: 310307.00003

13/25

Identification of the transcription start site by catalytic
autolabeling

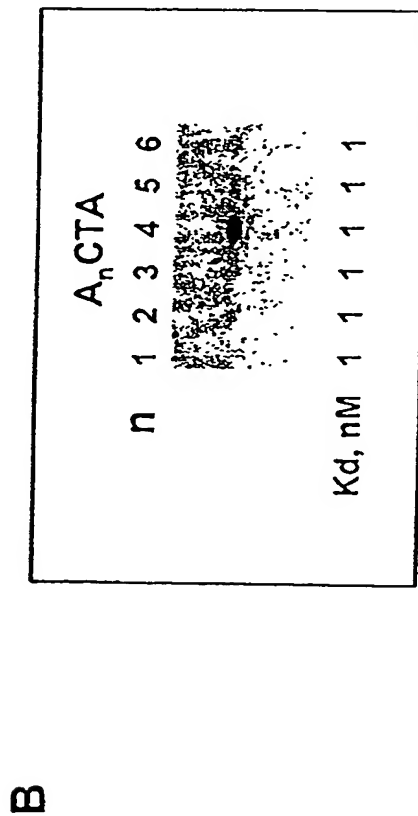
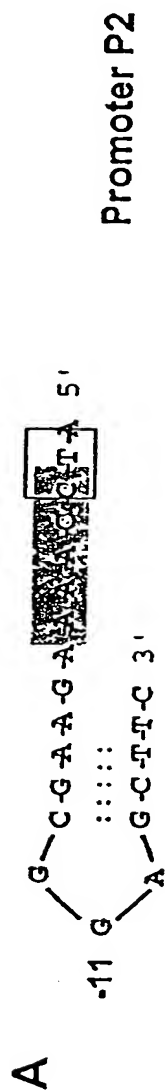


FIG. 13

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Docket Number: 310307.00003

14/25

UV crosslinking of mutant mini-vRNAPases
to promoter oligonucleotides

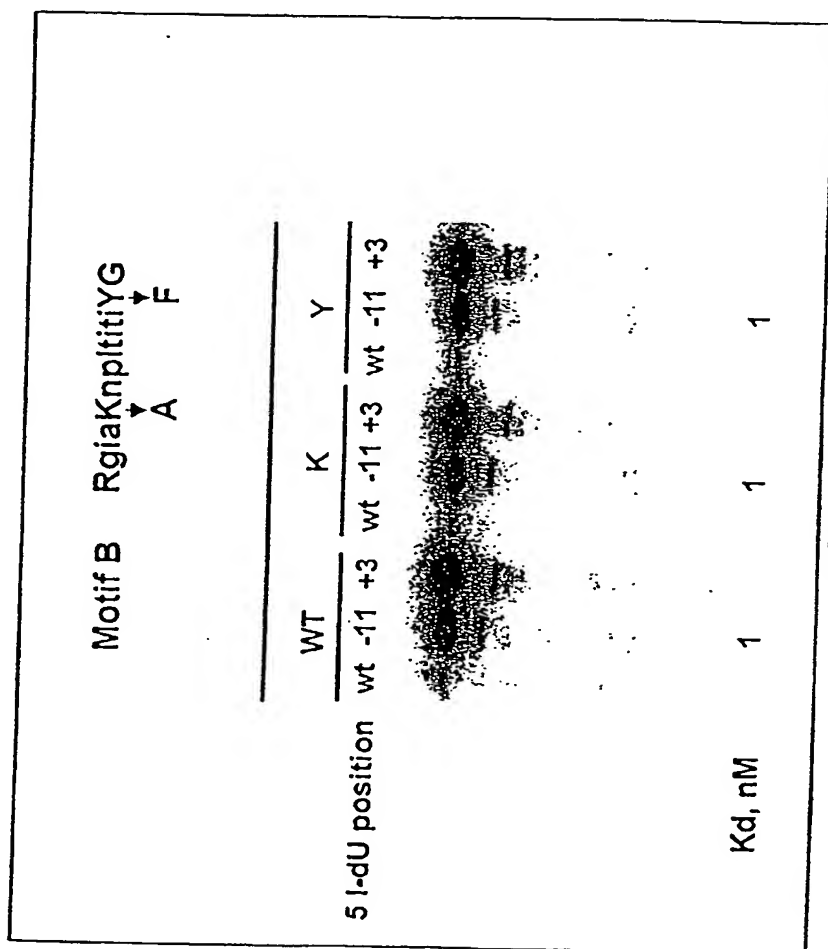


FIG. 14

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Docket Number: 310307.00003

15/25

Run-off transcription by mutant mini-vRNAPases

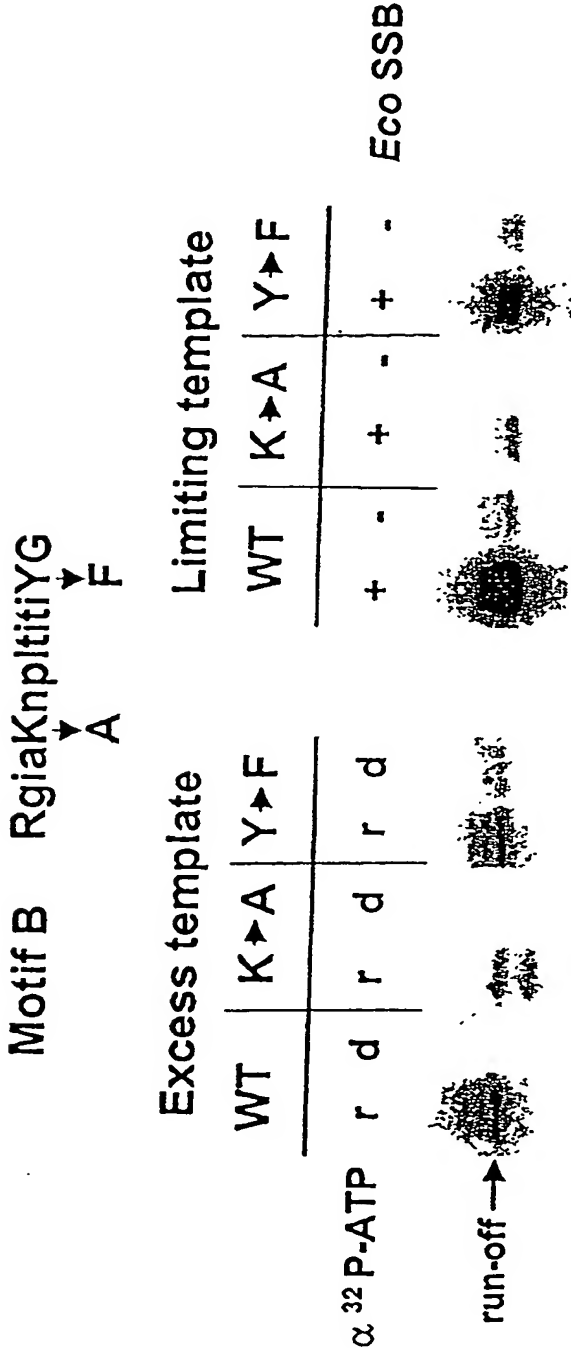


FIG. 15

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16/25

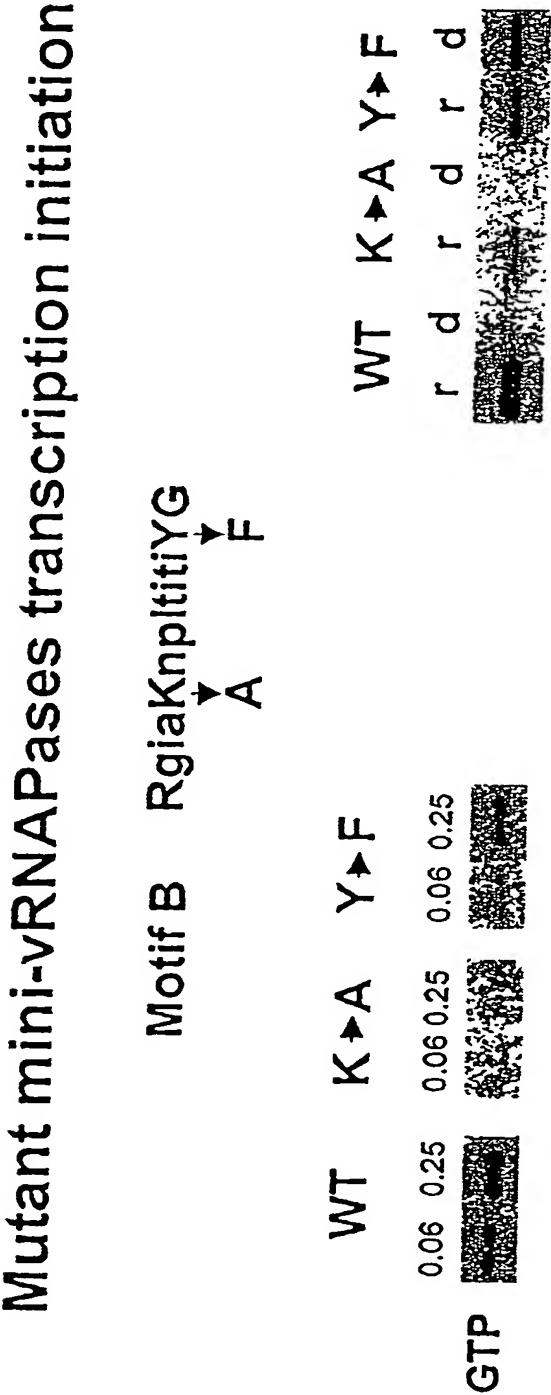


FIG. 16

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Application No.:

Docket Number: 310307.00003

17/25

Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

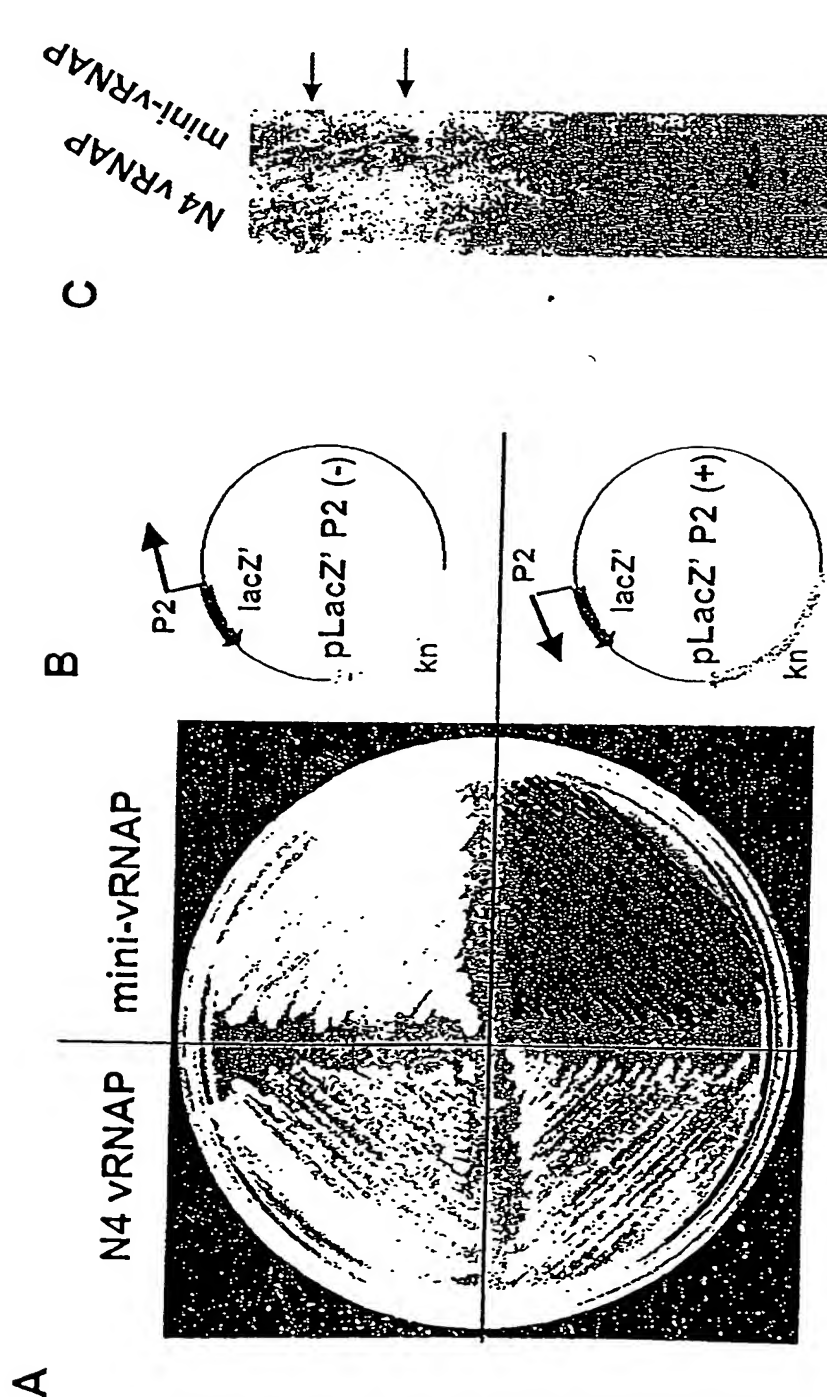


FIG. 17

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Application No.:

Docket Number: 310307.00003

18/25

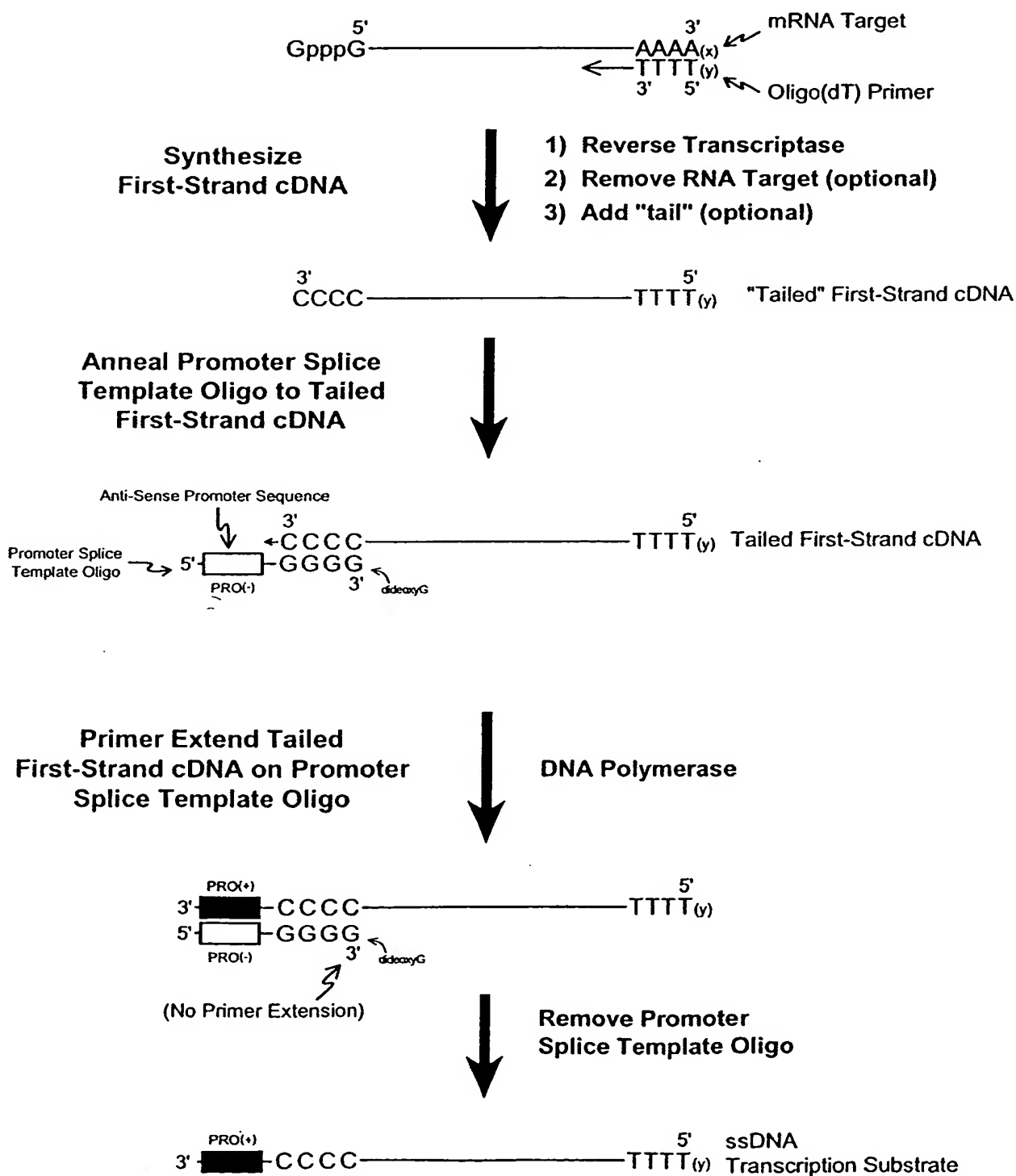


FIG 18

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19/25

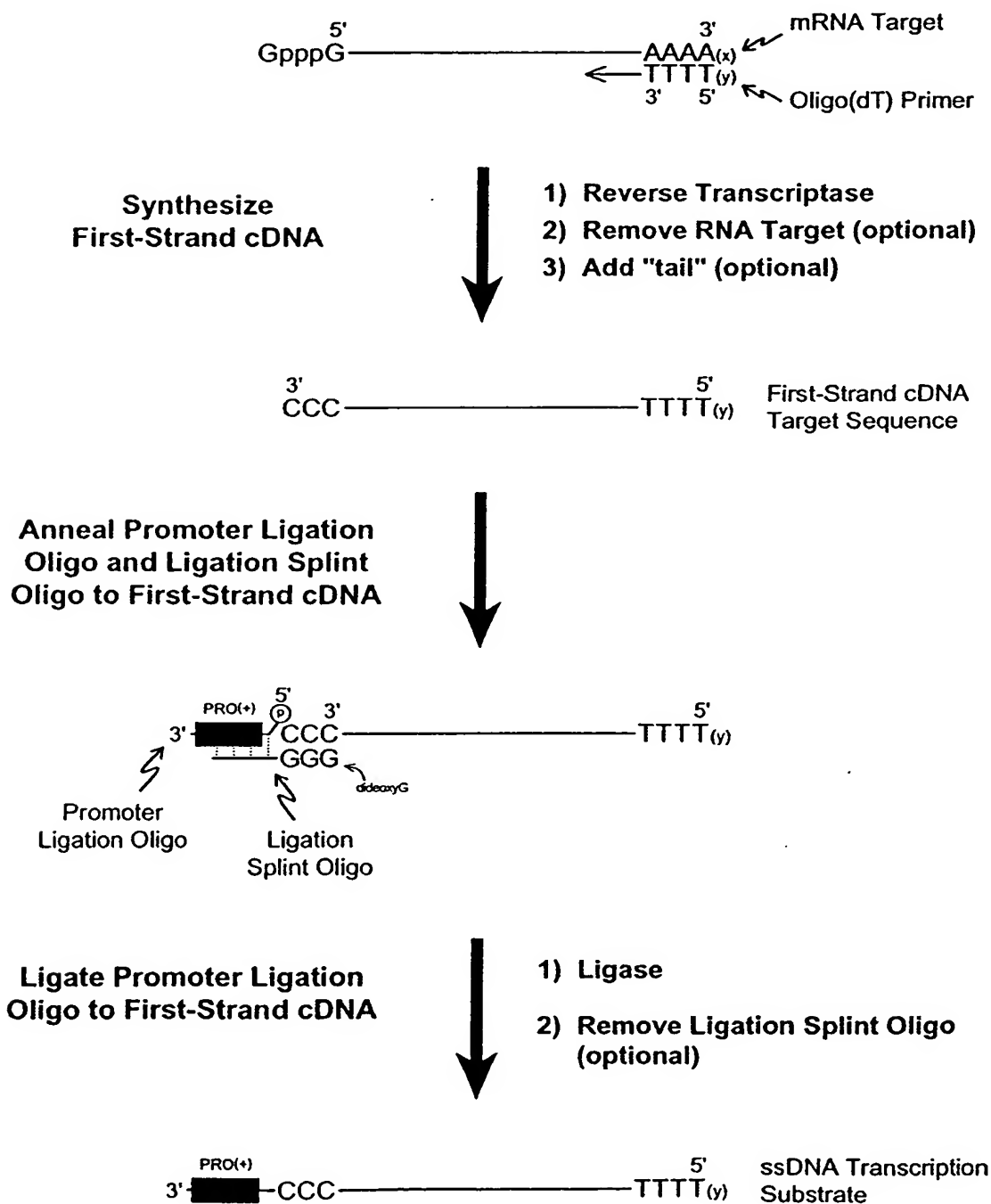


FIG 19

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20/25

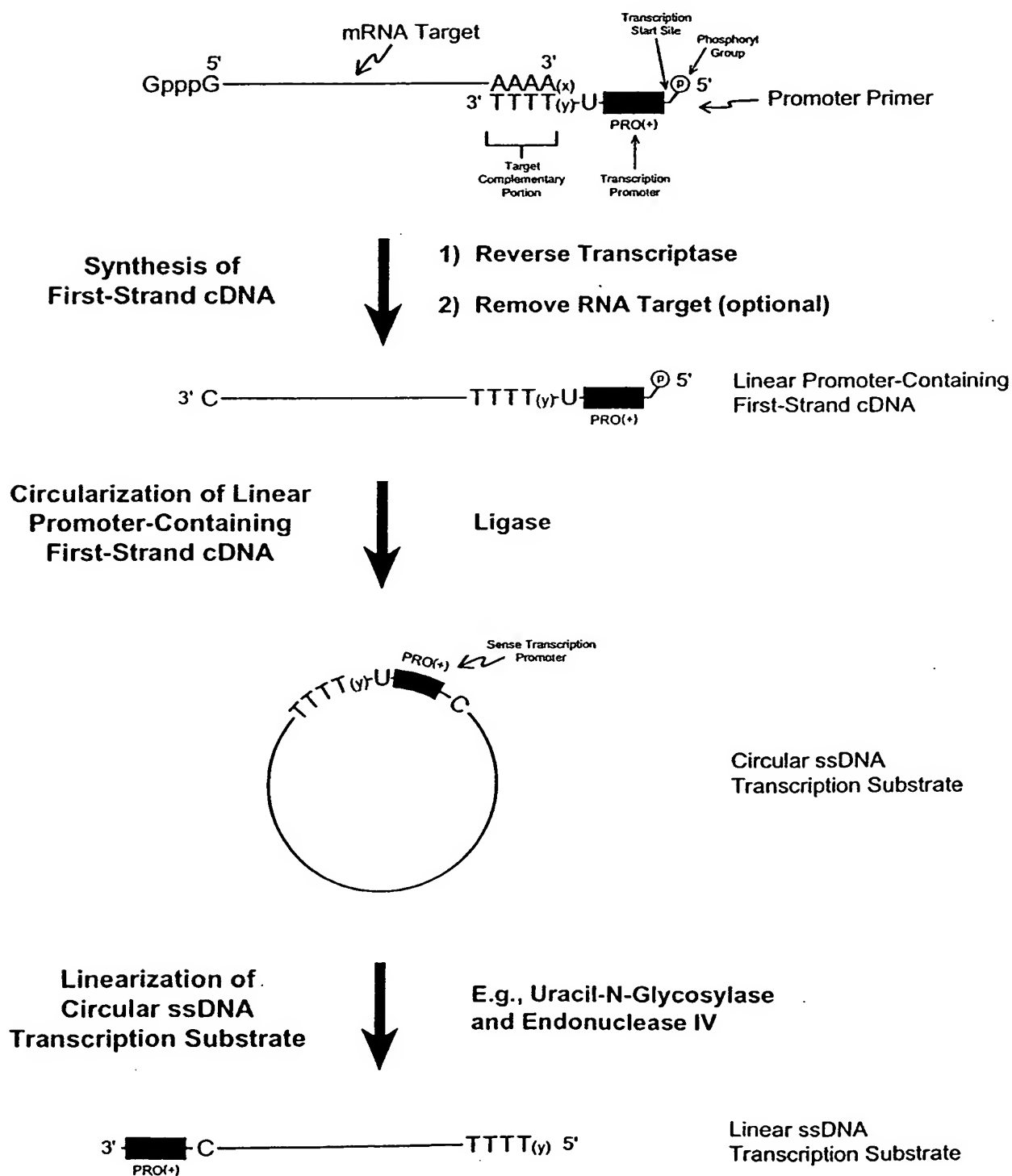


FIG 20

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21/25

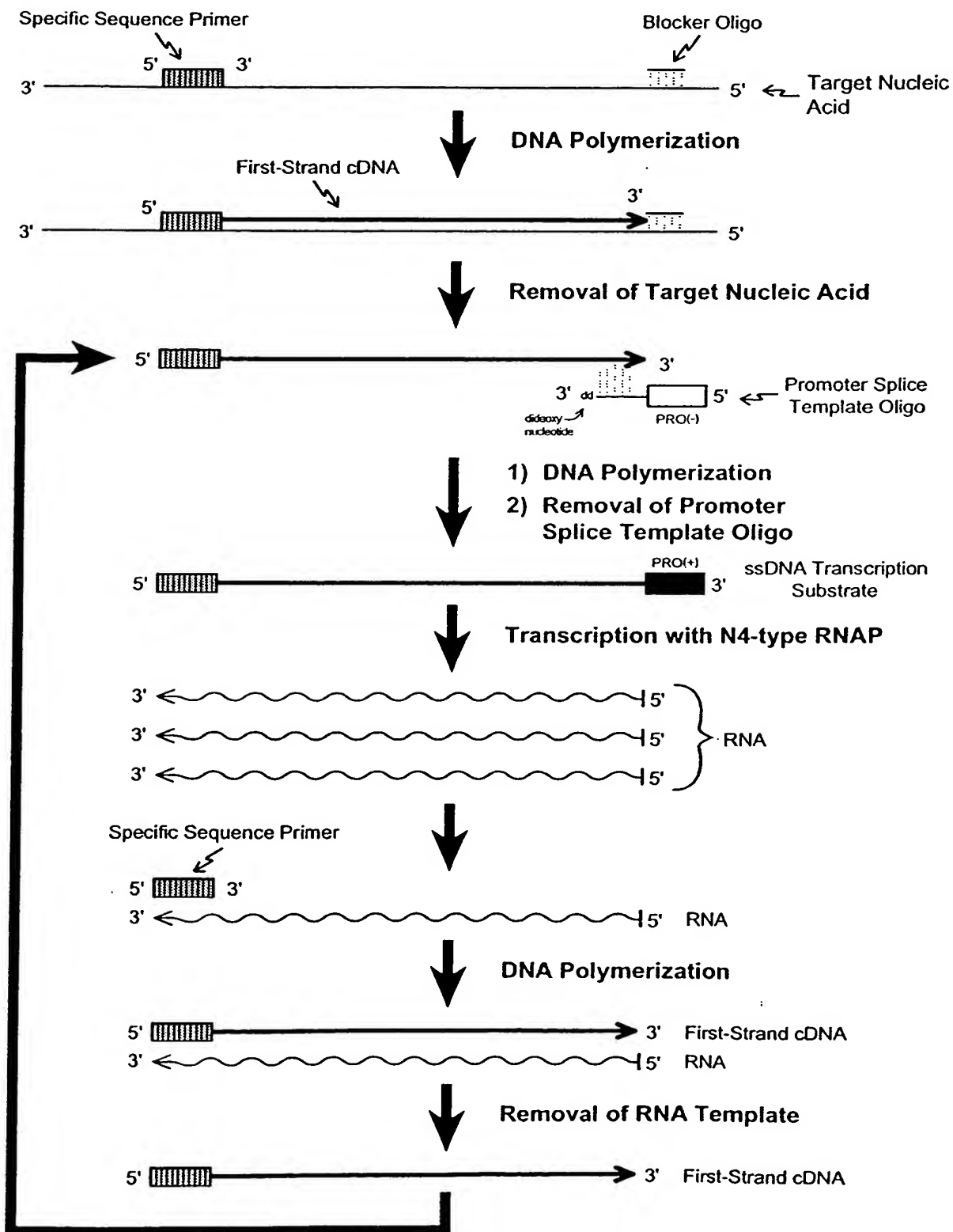


FIG 21

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22/25

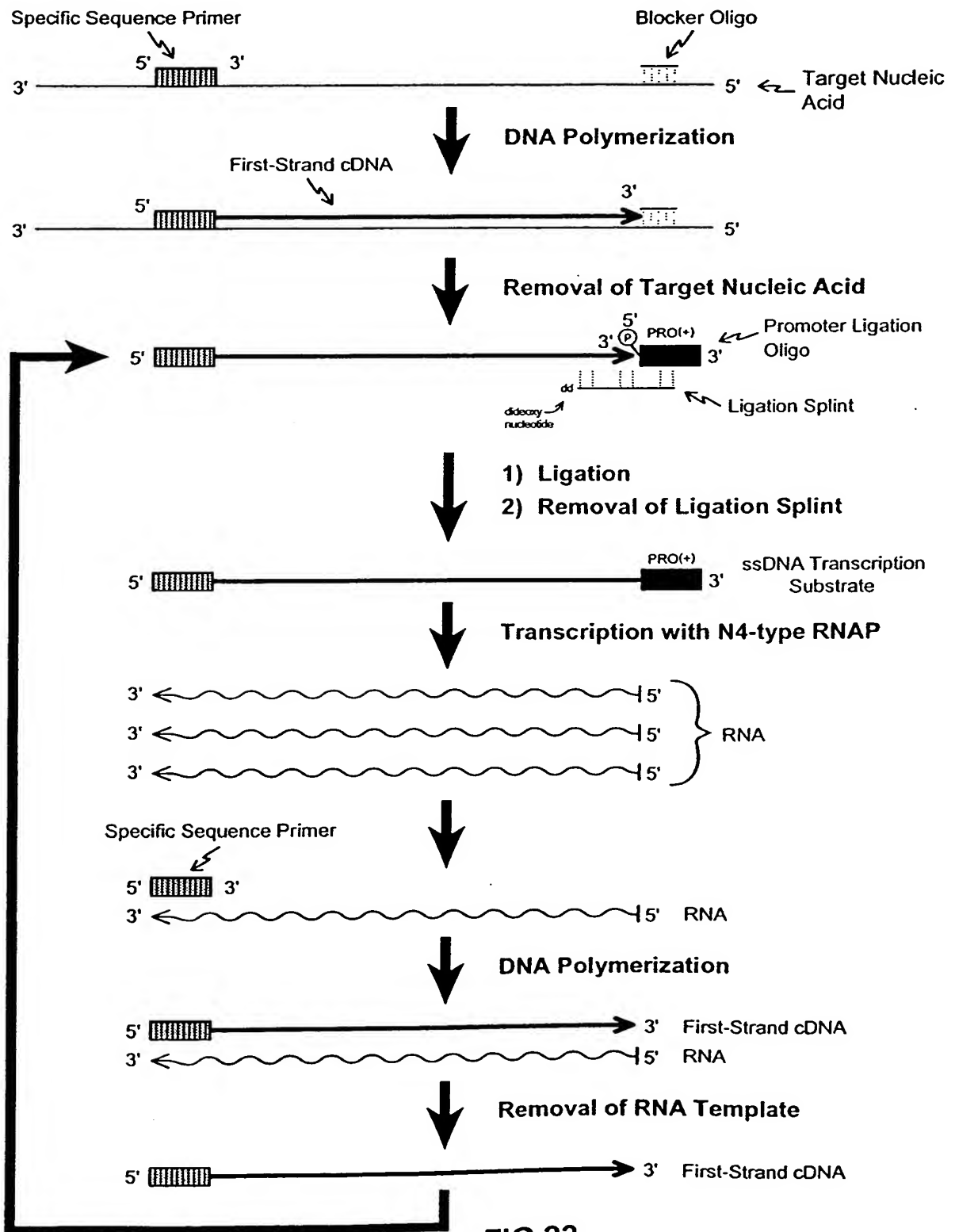


FIG 22

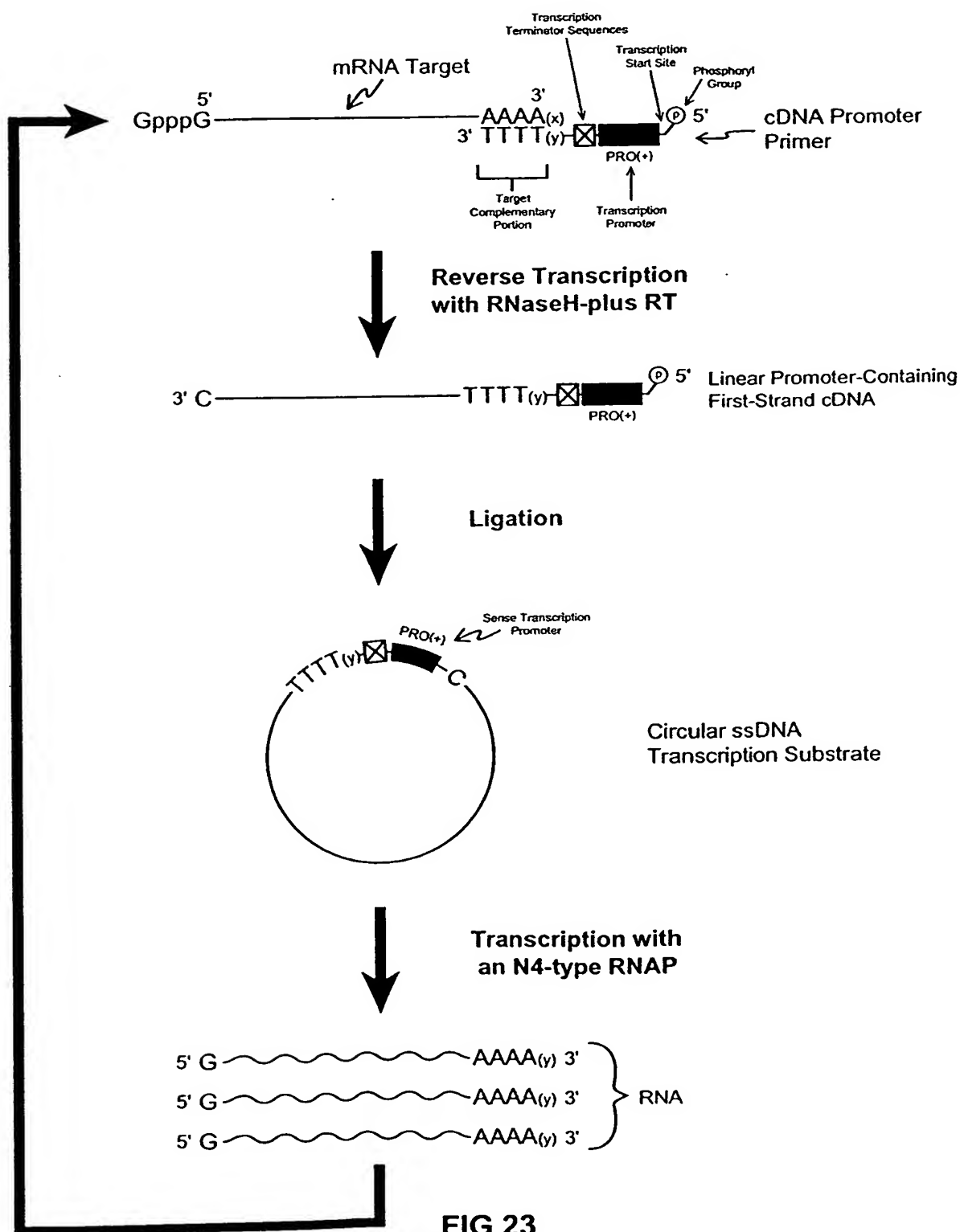
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23/25



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24/25

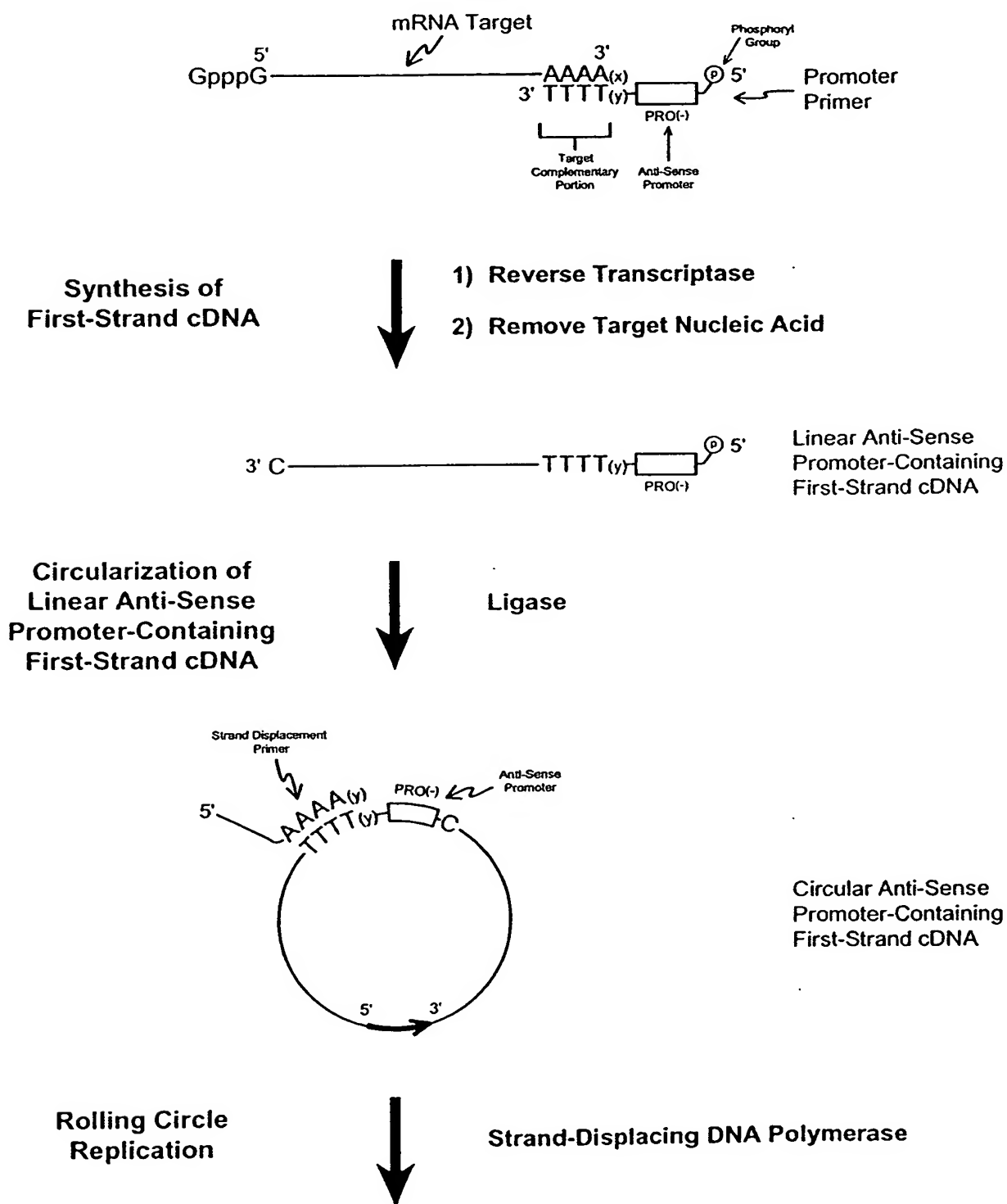


FIG 24 (continued on next page)

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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25/25

